A novel method to isolate native NifB-cofactor

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Shah et al (1994) described NifB-cofactor as the product of the NifB protein after accomplishing its *in vitro* purification in the *Klebsiella pneumonia* strain UN1217. Characterization of this small Fe-S cluster revealed its O_2-labile nature, greenish-brown appearance, EPR silence, and iron-only metal content. Later work showed that NifB-co was a FeMoco precursor that could be reconstituted *in vitro* using homocitrate, molybdenum and the NifEN scaffold protein (Curatti et al., 2007). Although the NifB-co structure is not fully understood, it was demonstrated that it comprises -at least- the 6Fe-9S core of FeMo-co coordinated with an interstitial light atom (George et al., 2008) that was later shown to be a carbon. Our inability to obtain fully homogeneous NifB-co preparations may be the reason why NifB-co structure is still eluding us, and it may have to do with sample alterations during the isolation procedure due to the effect of detergents and thiol reducing agents.

We have developed a new method to isolate intact NifB-co using a modified *K. pneumonia* UN1217 strain that overexpresses GST-tagged-NifX as a mean to hijack excess NifB-co. Interestingly, GST-NifX purifications present a greenish-brown colour due the presence of Fe-S cluster(s) which suggest that this strategy may be correct. Ethylene production after NifX-NifB-co reconstitution to FeMoco tested positive. We are currently testing NifX-NifB-co preparations for UV/visible, EPR, Mossbaüer, and protein crystallography to elucidate its structure.

References

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